## REMARKS

By this Supplemental Amendment, Applicants have amended claims 134, 150-152, 154 and 155, and added new claims 156-157. Independent claims 134 and 155 have been amended to recite "a RNA molecule" rather than "the RNA molecule." Dependent claims 150-152 and 154 have been corrected to recite "double-stranded DNA construct" to conform them to the language used in independent claim 134. Applicants maintain that the amendments made hereinabove do not raise any issue of new matter.

Accordingly, claims 134 to 157 are pending in the subject application.

This Supplemental Amendment also provides a substitute response to the rejections set forth in the November 3, 2008 Office Action. Applicants' arguments are in large part the same as those submitted in the May 4, 2009 Amendment, except that the arguments against the obviousness rejections now benefit from the support of a technical expert, Dr. Arthur D. Riggs. A Declaration of Dr. Arthur Riggs, which is attached hereto as **Exhibit 1**, has been provided by Dr. Riggs. The Declaration sets forth his expert viewpoint concerning the numerous unknowns about RNA interference at the time of the filing of the subject application, which made it impossible for one of ordinary skill in the art to predict the consequences of any deviation from what had been reported to have an RNA interference effect. Applicants request that the Examiner consider the remarks herein in lieu of the remarks in the May 4, 2009 Amendment.

**Support for New Claims**

The subject application is a continuation of U.S. Serial No. 10/759,841, filed January 15, 2004, which is a continuation of U.S. Serial No. 10/346,853, filed January 17, 2003, which is a continuation of U.S. Serial No. 09/100,812, filed June 19, 1998, now U.S. Patent No. 6,573,099 B2, issued June 3, 2003, which claims priority of Australian Provisional Patent Application No. PP2492, filed March 20, 1998 (the "Priority Application"). The new claims are fully supported in the disclosure of the Priority Application.

*Claims 150-152 and 154*

a) "double-stranded DNA construct"

The amendment inserting "double-stranded DNA" in claim 134 is supported, *inter alia*, by the numerous examples of "double-stranded" synthetic genes that are replete in the Priority Application. Specifically, a number of genetic constructs are described on page 28, line 14, to page 39, line 22 of the Priority Application. The genetic constructs described are ultimately derived from a double-stranded DNA plasmid, such as pCR2.1. *See, e.g.*, page 27, lines 1 to 8 of the Priority Application. Applicants attached as **May 4, 2009 Exhibit 1**[1] a map of plasmid pCR2.1, which is a commercially available starting plasmid for a number of the Examples. Additionally, the Priority Application describes blunt-ended fragments, which implies that

1 The label "**May 4, 2009 Exhibit _**" refers to an exhibit submitted with the May 4, 2009 Amendment in Response to November 3, 2008 Office Action. The label "**Exhibit _**" refers to an exhibit submitted herewith.

they are double stranded and have the potential to have an overhang. *See, e.g.*, page 37, lines 6 to 7 of the Priority Application.

*New Claims 156 and 157*

Support for new claims 156-157 may be found, *inter alia*, at page 3, lines 9 to 16; page 7, lines 5 to 7; page 8, lines 14 to 22; page 10, lines 15 to 21; page 16, lines 20 to 26; and page 18, lines 16 to 20 of the Priority Application.

**Claim Interpretation**

On page 5 of the November 3, 2008 Office Action, the Examiner has interpreted the configuration of Applicants' claimed double-stranded synthetic gene (currently amended to read "double-stranded DNA construct) set forth in the instant claims to be " a first structural gene sequence in the sense orientation relative to the target gene and a second structural gene sequence in the antisense orientation, making the second structural gene sequence complementary to the target gene." Applicants would like to clarify that each of the first and second "structural gene sequences" is comprised of double-stranded DNA. The Examiner's use of the word "complementary" in the above-referenced interpretation implies that the second structural gene sequence is single-stranded, which is incorrect. Claims 134 and 155 clearly describe a double-stranded DNA construct with a first double-stranded structural gene sequence, comprising 20-30 nucleotides in length identical in sequence to a region of a target gene, and a second double-stranded structural gene sequence comprising 20-30 nucleotides in length identical in sequence to and in an inverted orientation relative to the first

double-stranded structural gene sequence. Thus, the region of the resultant RNA derived from the first structural gene sequence will be in the sense orientation relative to the 20-30 nucleotides of the target RNA and the region of the resultant RNA derived from the second structural gene sequence will be in the antisense orientation and be complementary to the same 20-30 nucleotides of the target RNA.

**Information Disclosure Statement**

Applicants are concurrently submitting herewith a Supplemental Information Disclosure Statement, and respectfully request consideration of all items disclosed.

**Claim Objections: 37 CFR § 1.75(c)**

The November 3, 2008 Office Action objected to claims 146 to 149 under 37 CFR § 1.75(c) as allegedly of improper dependent form for failing to further limit the subject matter of claim 134.

In response, to clarify the invention, Applicants amended claim 134 to clarify that the length of the repeating sequence within the double-stranded DNA construct is only 20-30 nucleotides in length. For example, a double-stranded DNA construct within claim 134 would have a first structural gene sequence of 20 nucleotides and a repeated sequence, also of 20 nucleotides, inverted relative to the first structural gene sequence, with a stuffer fragment separating the two structural gene sequences. (*See, e.g.*, diagram "A", **May 4, 2009 Exhibit 2**). Because the stuffer fragment is not a "repeating sequence" it is not included in the 20-30 nucleotide size limitation and could therefore be any length. The restriction to 20-30 nucleotides relates to the

length of each of the first structural gene sequence and separately the second structural gene sequence forming a "repeating sequence" and does not limit the combined lengths of the first structural gene sequence, the stuffer, and the second structural gene sequence. Accordingly, claims 147 to 149 further limit the size of the stuffer fragment because they assign ranges of stuffer fragment lengths.

Similarly in response to the Examiner's remarks on page 3 of the November 3, 2008 Office Action with respect to claim 146. The interrupted palindrome is composed of the first structural gene sequence, the stuffer and the second structural gene sequence. The first and second structural gene sequences form a repeating sequence that is 20 to 30 nucleotides in length, and arranged as an inverted repeat. Therefore, the length of the sequence that is repeated is limited to 20 to 30 nucleotides and does not include the length of the stuffer.

**Written Description Rejection Under 35 U.S.C. § 112**

The November 3, 2008 Office Action objected to claim 134, and dependent claims 135, and 142 to 154, under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement because the claims contain subject matter that was not described in the specification. The Examiner referred to page 10, lines 15 to 21 of the Priority Application, and states that the structural genes of 20 to 30 nucleotides are a preferred embodiment targeting specific genes, including viral DNA or RNA polymerases, viral coat proteins, or visually-detectable genes involved in determining pigmentation, cell death or other external phenotypes. The Examiner purported that this section of the specification does not disclose the use of

structural genes of 20 to 30 nucleotides to target any gene, but only to target the specific classes of genes named. Because of this assumed limitation, the Examiner alleged that the disclosure of the specification is not commensurate in scope with the claimed invention.

In response, Applicants respectfully traverse.

The claims are commensurate in scope with the disclosure of the specification. The claimed invention is a process for reducing the expression of a "target gene." As stated in the specification, "the term 'target gene' shall be taken to refer to any gene, the expression of which is to be modified using the synthetic gene of the invention. Preferred target genes include, but are not limited to viral genes and foreign genes which have been introduced into the cell, tissue or organ or alternatively, genes which are endogenous to the cell, tissue or organ." (*See* page 7, lines 17 to 20 of the Priority Application). The claimed invention is aimed at reducing the expression of a "target gene" in mammalian cells. In the Examples described on pages 25 to 39 of the Priority Application, there are various examples of double-stranded DNA constructs that were designed to produce RNA that would reduce the expression of a "target gene" in mammalian cells. The DNA constructs designed for this purpose were derived from the commercially available mammalian expression vectors pEGFP-N1, pCMVLacI, pOPRSVI/MCS and pSVL. (*See* pages 25 to 26 of the Priority Application). The passage referred to by the Examiner on page 10, lines 15 to 21 of the Priority Application, describes "target genes" in mammalian cells and there is nothing in that passage that is not a target gene in a mammalian cell. The specific viruses named are all viruses that infect mammalian cells. Furthermore, the term "visually-detectable gene" is any

gene whose influence is visually detectable. Products of a visually detectable gene include RNA or protein, which can be detected visually using various technologies including Northern Blots, Quantitative Real-Time PCR, Western Blots, or other biochemical assays. Thus, a "visually-detectable gene" is merely a term the specification uses for a "target gene" in a mammalian cell.

Accordingly, Applicants respectfully submit that the passage referred to by the Examiner defines the minimum length of structural gene components aimed at target genes in mammalian cells to be 20 to 30 nucleotides. Because support *in haec verba* is not required, the specification fulfills the written description requirement for claim 134, and dependent claims 135, and 142 to 154.

If the Examiner still believes that the specification is not commensurate in scope with claim 134, Applicants have added new claim 155, which recites verbatim the subject matter from page 10 of the Priority Application cited by the Examiner.

**Claim Rejections Under 35 U.S.C. § 103(a) - Fire et al. Patent in view of in view of Agrawal et al., Gold et al., Kotin et al. and Chatterjee et al.**

The November 3, 2008 Office Action rejected claims 134 to 154 under 35 U.S.C. § 103(a) as allegedly unpatentable over Fire et al. (US 5,605,559) in view of Agrawal et al. (WO 94/01550), Gold et al. (US 5,270,163), Kotin et al. (US 5,580,703) and Chatterjee et al. (US 5,474,935). The Examiner's specific rationale is set forth on pages 6 through 10 of the November 3, 2008 Office Action.

Fire et al. Patent is not prior art to the claimed invention

As Applicants pointed out previously, Fire et al. Patent is not prior art to the subject application. The amended claims herein are entitled to the priority of the March 20, 1998 filing date of Australian Provisional Patent Application No. PP2492. Fire et al. Patent issued from an application submitted to the United States Patent and Trademark Office on December 23, 1998, *i.e.* after the priority date of the subject application.

Fire et al. Patent claims the benefit of U.S. Provisional Application No. 60/068,562, filed December 23, 1997 ("Fire et al. Provisional"). However, Fire et al. Provisional discloses less than Fire et al. Patent. Applicants attach hereto as **May 4, 2009 Exhibit 3** a copy of Fire et al. Patent marked-up to show differences from Fire et al. Provisional. Any rejection which relies on disclosure not in Fire et al. Provisional is improper.

In the November 3, 2008 Office Action, the Examiner stated that

Applicants did not provide specific arguments that illustrated the relevance of the differences between the Fire et al. Provisional and the Fire et al. Patent. In response, Applicants respectfully submit that it is not Applicants' responsibility to explain how a rejection may be asserted based on the disclosure of the Fire et al. Provisional; rather, the initial burden is on the Examiner to set forth a rejection based on disclosure that predates Applicants' priority date. The Examiner has not met this initial burden at least because the Examiner has relied on disclosure which does not predate Applicants' priority date. Specifically, at least the following portions of the rejection rely on disclosure which is not prior art:

- On page 5, line 3, of the November 6, 2007 Office Action the Examiner stated "Fire et al. teach at columns 21-22 that a single promoter can be used to express an inverted duplication of a self-complementary dsRNA … ." This information is not disclosed in the Fire et al. Provisional (*See,* **May 4, 2009 Exhibit 3** difference 168).

- On page 7, line 3, of the November 3, 2008 Office Action the Examiner stated "At column 10 [of the Patent] Fire et al. teach that viruses can be targeted, including HIV." This information is not disclosed in the Fire et al. Provisional (*See,* **May 4, 2009 Exhibit 3** difference 132).

Because the foregoing information was disclosed after the Applicants' priority date, it cannot be relied upon to reject Applicants' invention. Consequently, the rejections which rely on this disclosure are improper and must be withdrawn.

Aside from the aforementioned deficiency, the rejections set

forth in the November 3, 2008 Office Action are also deficient in the manner discussed hereinbelow.

## I. The Obviousness Rejections Are Unsupported by the Evidence and Controlling Law

The determination of whether a claimed invention is obvious requires an analysis according to the framework of *Graham v. John Deere Co.*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966) (**May 4, 2009 Exhibit 4**). *See,* M.P.E.P. § 804(II)(B)(1). The *Graham* analysis requires the following factual inquiries:

a. determine the scope and content of the combined teaching of the prior art;

b. determine the differences between the combined teaching of the prior art and the claims at issue;

c. determine the level of ordinary skill in the pertinent art; and

d. evaluate any objective indicia of nonobviousness (secondary considerations).

The Supreme Court of the United States most recently reaffirmed the *Graham* analysis in *KSR International Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 82 U.S.P.Q.2d 1385, 1391 (2007) (**May 4, 2009 Exhibit 5**). The Supreme Court has continually cautioned against slipping into hindsight reconstruction. The *Graham* Court cautioned that it is necessary "to guard against slipping into use of hindsight and to resist the temptation to read into the prior art the teachings of the invention in issue." *Graham*, 383

U.S. at 36, 148 U.S.P.Q. at 474 (internal quotations omitted). The *KSR* Court reiterated the need for a fact finder to be aware "of the distortion caused by hindsight bias" and to "be cautious of arguments reliant upon ex post reasoning." *KSR*, 82 U.S.P.Q.2d at 1397.

Factors such as uncertainty and lack of predictability in the field at the time of the invention must be considered. *See, e.g. KSR,* 82 U.S.P.Q.2d at 1396. At least some degree of predictability is required in the prior art to render an invention obvious. An invention resulting from exploring a new technology where the prior art gave only general scientifically untested guidance on how to achieve the result is not considered "obvious to try." *See, In re O'Farrell*, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988), attached hereto as **Exhibit 2**. Even if there was a general suggestion or motivation to attempt to produce the invention, uncertainty and lack of predictability in the field will render the invention patentable and not obvious. *See,* M.P.E.P. § 2143.02; *In re Vaeck*, 947 F.2d 488, 495, 20 U.S.P.Q.2d 1438, 1444 (Fed. Cir. 1991); *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1207-08, 18 U.S.P.Q2d 1016, 1022-23, (Fed. Cir. 1991) (**May 4, 2009 Exhibits 6 and 7**, respectively) (Holding invention non-obvious even though it was "obvious to try" because lack of predictability in the biotechnology field eliminated reasonable expectation of success). Consideration of these factors is necessary when analyzing whether an invention is obvious; as the Supreme Court explained in *KSR,* one "must ask whether the improvement is more than the predictable use of prior art elements." *KSR,* 82 U.S.P.Q.2d at 1396 (emphasis added).

The analysis is the same regardless of where in the prior art the elements are disclosed. The patentability of a claim to a species or subgenus embraced by a single prior art generic disclosure should be analyzed no differently than any other claim for purposes of 35 U.S.C. 103. *See, e.g. Ortho-McNeil Pharmaceutical, Inc v. Mylan Laboratories, Inc.*, 520 F.3d 1358, 86 U.S.P.Q. 1196 (Fed. Cir. 2008); *In re Papesch*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963); *In re Brouwer*, 77 F.3d 422, 37 U.S.P.Q.2d 1663 (Fed. Cir. 1996); *In re Ochiai*, 71 F.3d 1565, 37 U.S.P.Q.2d 1127, (Fed. Cir. 1995); *In re Baird*, 16 F.3d 380, 29 U.S.P.Q.2d 1550 (Fed. Cir. 1994); (**May 4, 2009 Exhibits 8 to 12**, respectively) and M.P.E.P. § 2144.08 (Rev. 6, Sept. 2007). When determining whether a claimed invention is patentable, the relevant inquiry is not whether a particular difference between the prior art and the claims would have been obvious, but whether the claimed invention as a whole would have been obvious. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 U.S.P.Q. 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782, 218 U.S.P.Q. 698 (Fed. Cir. 1983) (**May 4, 2009 Exhibits 13 and 14**, respectively).

As the Federal Circuit instructively explained in *Eisai Co. Ltd. v. Dr. Reddy's Laboratories, Ltd.*, 533 F.3d 1353, at 1359 (Fed. Cir. 2008), attached hereto as **Exhibit 3**.

> the obviousness inquiry must rely on evidence available 'at the time' of the invention, *see Takeda,* 492 F.3d at 1356 n. 2[)]. The Supreme Court's analysis in *KSR* thus relies on several assumptions about the prior art landscape. First, *KSR* assumes a starting reference point or points in the art, prior to the time of invention,

> from which a skilled artisan might identify a problem and pursue potential solutions. Second, *KSR* presupposes that the record up to the time of invention would give some reasons, available within the knowledge of one of skill in the art, to make particular modifications to achieve the claimed compound. *See Takeda,* 492 F.3d at 1357 ('Thus, in cases involving new chemical compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound.'). Third, the Supreme Court's analysis in *KSR* presumes that the record before the time of invention would supply some reasons for narrowing the prior art universe to a 'finite number of identified, predictable solutions,' 127 S.Ct. at 1742. In *Ortho-McNeil Pharmaceutical, Inc. v. Mylan Laboratories, Inc.,* 520 F.3d 1358, 1364 (Fed. Cir. 2008), this court further explained that this 'easily traversed, small and finite number of alternatives ... might support an inference of obviousness.' To the extent an art is unpredictable, as the chemical arts often are, *KSR*'s focus on these 'identified, predictable solutions' may present a difficult hurdle because potential solutions are less likely to be genuinely predictable. (Emphasis added) (*See, Takeda* attached hereto as **Exhibit 4** and *Ortho-McNeil Pharmaceutical, Inc.* **May 4, 2009 Exhibit 8.**

In the instant case, instead of exogenously injecting a prepared double-stranded RNA, which was the only reported example of RNA interference in the prior art at the time, Applicants' claimed invention recites a process producing in the nucleus of a

mammalian cell a hairpin RNA. *See,* Declaration of Dr. Arthur Riggs ("Riggs Decl."), attached hereto as **Exhibit 1**, ¶¶ 6, 11, 14 and 15. Such a method of endogenously expressing hairpin RNA for delaying repressing, or otherwise reducing expression of a target gene in an animal cell was not described by the prior art. The combined effects of changing to endogenous production of a RNA different from that reported to work by the only example of RNA interference (Fire et al.) prior to Applicants' invention could not be predicted. *See,* Riggs Decl. ¶¶ 14 and 15. In yet a further departure, Applicants' claims requires the DNA construct to have a structural gene sequence of 20-30 nucleotides in length to produce a double-stranded RNA that was an order of magnitude shorter than the 299-1033 nucleotide length double-stranded RNA injected by Fire et al. *See,* Riggs Decl. ¶ 11 and 24. Having no information about the mechanism of RNA interference, and knowing that the mechanism is unlikely to resemble the gene silencing mechanism of antisense, one of ordinary skill in the art prior to Applicants' invention had no basis to predict whether the selection proposed by the Examiner, as a whole, would work. *See,* Riggs Decl. ¶¶ 6-8 and 13 to 26.

As established by factual evidence from the relevant time, Applicants' claimed invention is more than merely the predictable use of prior art elements. Predictability in achieving a result specified in a patent claim through assembly of "known" components was a critical element of the Supreme Court's *KSR* decision, and subsequent Federal Circuit decisions. Indeed, the Court in *KSR* emphasized the importance of asking whether or not a particular combination of references would lead to a predictable solution to a problem. *See, e.g., KSR,* 82 U.S.P.Q.2d at 1397. Likewise, the Federal Circuit emphasized asking whether a particular solution was "genuinely predictable." *See, e.g., Eisai*

*Co. Ltd. v. Dr. Reddy's Laboratories, Ltd.*, 533 F.3d at 1359. RNA interference and particularly its mechanism were a mystery <u>at the time</u>; as such there was prior to Applicants' invention hardly anything predictable about the "solution" now being proposed by the Examiner.

When ascertaining whether an invention is obvious, "[a] factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." *KSR International Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 82 U.S.P.Q.2d 1385, 1397 (2007). Applicants respectfully submit and show herein that the obviousness rejections of record hint of hindsight bias and do rely on *ex post* reasoning.

**II Application of the Controlling Legal Precedent to the Facts from the Relevant Time.**

**1. <u>Scope and Content of the Prior Art</u>**

i) The mechanism for RNA interference was unknown in March 1998, making genuine predictions in the field impossible

The primary reference, the Fire et. al. Provisional, reported that exogenous delivery of certain double-stranded RNA to cells by injection into the body cavity of *C. elegans* resulted in the inhibition of a targeted gene. The double-stranded RNA used was produced outside of the organism as separate strands, subsequently annealed, and was sequence specific to the target gene over lengths from 299 to 1033 nucleotides. This was the first report of RNA interference. *See,* Riggs Decl. ¶ 6.

The Fire et al. Provisional fails to describe a mechanism to explain the reported results. However, the Fire et al. Provisional explains at length that the mechanism, whatever it may be, is unrelated to known approaches for interfering with gene expression. Specifically, on pages 2-5, the Fire et al. Provisional discloses that the unknown mechanism is distinct from that of antisense interference, from that of triple-helix interference, and from that of co-suppression approaches. *See,* Riggs Decl. ¶ 6.

ii) The Fire et al. Provisional contains common boilerplate purporting to generalize the only reported results of RNA interference

Despite teaching that the mechanism underlying the RNA interference phenomenon is different from all known phenomena, the Fire et al. Provisional contains common boilerplate purporting to generalize the reported results. Portions of the generic disclosure which have been relied upon by the Examiner[2] or which are relevant for later analysis of differences recited in the pending claims are summarized below.

Although the technique was only reported to work in *C. elegans*, the Fire et al. Provisional made a broad statement that the "cell with the target gene may be derived from or contained in any organism. The organism may [be] a plant, animal, fungus, or yeast" (page 11, lines 3 to 4) and goes on to list multiple species of plants, vertebrate animals, and invertebrate animals

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[2] Notably, the obviousness rejections pick and choose from this generic boilerplate but do not analyze the technical consequences of making the modifications to the experiments reported in the Fire et al. Provisional.

(page 11, lines 5 to 11). Except for the reported work in *C. elegans*, there was skepticism in the prior art about similar results in other organisms, as evidenced by statements of those of skill in the art at the time, such as "Whatever the mechanism might be, dsRNA-mediated inhibition of gene expression will provide a useful alternative for working out gene function in *C. elegans* and, maybe, in other animals and plants." (Emphasis added). *See,* Wagner R.W. and Sun L., Nature, 1998, **May 4, 2009 Exhibit 18** and Riggs Decl. ¶¶ 6-7.

The Fire et al. Provisional describes that the "RNA may be introduced directly into the cell (*i.e.*, intracellularly) or extracellularly." (Page 12, lines 1 to 2 of the Fire et al. Provisional). Importantly, "intracellularly," as used there, refers to introduction by injection as the needle delivers the RNA inside the cell as opposed to delivering RNA to the extracellular space or body cavity. The Fire et al. Provisional discloses multiple methods for introducing the RNA to the target cells, including "injection … , bombardment by particles covered by the RNA, soaking the cell or organism in a solution of the RNA, or electroporation of cell membranes with the RNA" (page 12, lines 7 to 10).

The Fire et al. Provisional discloses that "A viral vector packaged into a viral particle would accomplish both efficient introduction of an expression vector into the cell and transcription of RNA encoded by the expression vector" (page 12, lines 10 to 12). This disclosure encompasses but does not describe a wide range of potential options including the following: (1) RNA retroviral vectors whose genomes are integrated into the host after reverse transcription and are expressed in the nucleus, including Murine leukemia virus and

Lentiviruses; (2) single-stranded positive sense RNA virus vectors whose genes are expressed in the cytoplasm, including Sindbis virus, Semliki Forest virus, Poliovirus, and Kunjin virus; (3) single-stranded negative sense RNA virus vectors whose genes are expressed in the cytoplasm, including Influenza virus, Rabies virus, Vesicular stomatitis virus, and Sendai virus; and/or (4) double-stranded DNA virus vectors whose genes are expressed in the nucleus, including SV40 virus, Herpes Simplex Virus, Papillomavirus, Epstein Barr Virus, Adenovirus, Adeno-Associated virus and Baculovirus.

The Fire et al. Provisional does, however, teach that "[p]hysical methods of introducing" the RNA are "preferred". Page 12, line 7. Thus, the Fire et al. Provisional discloses all possibilities of known methods of introducing the RNA into a cell, but <u>guides the reader that physical methods</u>, i.e. not viral vector packaged into a viral particle type of methods, <u>are the "preferred" methods for RNA interference</u>.

Except for the delivery of double-stranded RNA to the gonads and the body cavity of *C. elegans*, all other delivery methods were untested and unpredictable prior to Applicants' invention. *See,* Riggs Decl. ¶¶ 15-25.

Each class of the foregoing options presented by the Fire et al. Provisional is not in any way connected to any other class of options. Other than the teaching of a preference for the use of physical delivery methods over other methods, no interrelationship is disclosed, for example, with regard to what type of delivery method could be used with which type of RNA molecule. The elements listed in the Fire et al. Provisional merely include all the possible eukaryotic cell types, all the

common delivery methods for nucleic acids, and all methods of producing RNA, that were known to one skilled in the art at that time. *See,* Riggs Decl. ¶ 7

iii) Agrawal et al., Chatterjee et al., Gold et al. and Kotin et al. are from non-analogous arts and offer no insight into the predictability of Applicants' selection

The Fire et al. Provisional is the only cited RNA interference reference cited by the Examiner. Agrawal et al. and Chatterjee et al. relate to antisense art. Initially, the propriety of the Examiner's combination is suspect because Fire et al. explicitly teach that "[a] simple antisense model is not likely: annealing between a few injected RNA molecules and excess endogenous transcripts would not be expected to yield observable phenotypes." *See,* Fire et al. Letter to Nature, **May 4, 2009 Exhibit 15**; and pages 2-5 of the Fire et al. Provisional. The Examiner's combination of Agrawal et al. and Chatterjee et al. with the Fire et al. Provisional is at the least not suggested by prior art; and in fact it is a particularly improper case of hindsight reconstitution in view of the extensive discussion on pages 2 to 5 of the Fire et al. Provisional distinguishing RNA interference from antisense methods.

The other references, Gold et al. and Kotin et al., relate to protein biochemistry and virology, respectively, which are not in the scope of Applicants' invention. Thus, the combination of elements selected from the secondary references with the elements selected from the Fire et al. Provisional is improper because it relies on current knowledge of the RNA interference mechanism, not knowledge prior to Applicants' invention.

Despite their contention that the combination proposed by the Examiner relies on *ex post* reasoning and is consequently improper, for completeness of the record, Applicants show below that the combination fails to teach Applicants' claimed process with the required expectation of success.

<u>Agrawal et al.</u>

Agrawal et al. describe self-stabilized antisense oligonucleotides that comprise a target hybridizing region and a self-complementary region. *See* Agrawal et al. page 8, lines 22 to 25. The target hybridizing region is "preferably" from "about 8 to about 50 nucleotides in length" (*See* Agrawal et al. page 9, line 36 to page 10, line 1) and the self-complementary region is "about 4 or more base-pairs" but, in a preferred embodiment, "about 10 intramolecular base-pairs" (*See* Agrawal et al. page 15, lines 21 to 26). The self-complementary region could "involve every nucleotide of the oligonucleotide" and in this instance, the self-complementary region would be "about 50 nucleotides or less." *See* Agrawal et al. page 15, lines 26 to 30. Agrawal et al. teach that the "loop" formed by the self-complementary region should involve the "3'-most nucleotides" to protect the 3' end from endonucleases. *See* Agrawal et al. page 15, lines 20 to 26. Agrawal et al. teach that the oligonucleotides could be synthesized in vitro by chemical methods and they may contain modified linkages. *See* Agrawal et al. page 14, lines 11 to 35. The target hybridizing regions of the oligonucleotides could be complementary to nucleic acid sequences from viruses, pathogenic organisms, or cellular genes. *See* Agrawal et al. page 10, line 14 to page 13, line 4.

Chatterjee et al.

Chatterjee et al. describe the use of Adeno-associated virus (AAV) vectors, which integrate into the genome of the host, to express antisense oligonucleotides or dominant-negative proteins that will down regulate the expression of targeted viral or cellular genes. Specifically, Chatterjee et al. target the 5' untranslated regions of human immunodeficiency virus (HIV) or herpes-simplex virus (HSV) RNA molecules. Chatterjee et al. also discuss possible mechanisms for antisense RNA including "the higher the molar ratio of antisense to sense transcripts, the greater the inhibitory effect" (Chatterjee et al. column 9, lines 29 to 31). This statement is discordant with the observations of Fire at al., further supporting the fact that antisense art and RNA interference occurred via two distinct mechanisms.

*Gold et al.*

Gold et al. describe a method to identify nucleic acid ligands for protein molecules. They transcribe DNA templates in vitro to produce single-stranded RNA, which contains randomized, contiguous regions of nucleotides for the purpose of interacting with proteins. In various figures of the specification, this randomized region corresponds to the loop of a RNA hairpin. The various RNAs are mixed with the target protein to promote RNA-protein interactions and the RNA molecules that bind are selected for another round of testing. They are amplified and the process is repeated. After a number of rounds of selection, Gold et al. are able to select for the sequence that most strongly interacts with the target protein.

*Kotin et al.*

Kotin et al. describe the isolation of a 4 kb long nucleic acid fragment that corresponds to the integration site of human adeno-associated virus (AAV). Also included within the fragment is a structural gene with a TATA-less promoter. Kotin et al. clearly state that this nucleic acid sequence can be "employed as a probe, in conjunction with gene therapy, to identify successful integration of AAV" (*See* Kotin et al. column 2, lines 34 to 37). However, while Kotin et al. discuss the "potential advantages" of an AAV-based vector system, they do not describe an AAV-based vector and instead refer to the "proposed use" of AAV as a vector. (*See,* Kotin et al. column 2, lines 33 to 34).

In summary, none of the secondary references, even when combined with Fire et al., suggest the Applicants' claimed invention. As Fire et al. Provisional acknowledged, the antisense art could not contribute any information that would have assisted one of ordinary skill in the art to select the elements recited in the pending claims or to provide an expectation of such selection being successful. Furthermore, a discussion of nucleic acid ligands that act as protein inhibitors is irrelevant and knowledge of such technology would not have aided one of ordinary skill in the art in the development of the double-stranded DNA constructs for RNA interference described in the present claims. The knowledge in the nascent field was simply not dependable. *See,* Riggs Decl. ¶¶ 8 and 9.

## 2. **Differences Between the Prior Art and the Pending Claims**

i) The Examiner with the benefit of hindsight has selected from the prior art an unpredictable possibility for inducing RNA interference.

The claimed invention is based on a selection of certain elements disclosed in the prior art and arranged in a manner not at all disclosed in the prior art. More specifically, the pending claims recite the results of the selection and combination of elements as summarized in the following list, and explained in detail thereafter:

a) *Endogenous delivery.* The constructs used in the methods as claimed are designed to deliver double-stranded RNA to the target cell by producing the RNA in the nucleus of mammalian cells. This approach differs from that reported to work by Fire et al., who produced double-stranded RNA in vitro and then injected the RNA into the gonad, body cavity or cytoplasm of *C. elegans* intestinal cells. Although multiple options for introducing the RNA to the target cells are suggested in the Fire et al. Provisional, it is clear that the "physical methods of introducing" the RNA were preferred (*See,* Fire et al. Provisional page 12, lines 7 to 10). Chatterjee et al. use AAV vectors to express antisense RNA in the nucleus of target cells, but since the mechanism of antisense RNA differs from that of RNA interference, this disclosure has no relevance to predicting effects of its combination with the Fire et al. Provisional. Kotin et al. discuss the "potential advantages" of AAV-based vectors, but never

describe the construction or use of such vectors, let alone for reducing gene expression, and consequently teach even less than Chatterjee et al. Therefore, neither the Fire et al. Provisional, nor the cited references from the art at the time, either alone or in combination, provided a motivation to select endogenous delivery of the double-stranded RNA for RNA interference. More importantly, the prior art provided no guidance to one of ordinary skill in the art prior to the filing of the subject application for predicting the effects of such selection. *See,* Riggs Decl. ¶¶ 14 to 22.

b) *RNA hairpin structure.* Fire et al. recognized that "RNA structure was responsible for [its] inhibitory activity" (*See* page 14, lines 24 to 25) and they teach that double-stranded RNA composed of <u>two, separate</u> RNA strands is capable of inhibiting gene expression. Applicants' claims recite methods that use double-stranded DNA constructs that would produce a <u>single</u> RNA strand in the nucleus designed to fold over onto itself to form a duplexed hairpin structure. This structure differs from that of Fire et al.'s double-stranded RNA because it will have a loop of un-base-paired nucleotides on one end of the duplex. One of ordinary skill in the art at that time had no basis to select this different RNA structure. More importantly, the prior art provided no guidance to one of ordinary skill in the art prior to the filing of the subject application for predicting how such a selection would affect the ability of the DNA constructs to produce RNA to delay, repress or otherwise reduce the expression of a target gene in a mammalian cell. *See,* Riggs Decl. ¶ 23.

The Examiner stated that Agrawal et al. teach a self-stabilized oligonucleotide. However, the oligonucleotides of Agrawal et al. differ from those of the claimed invention. Agrawal et al. define two regions of a self-complementary oligonucleotide (the "target hybridizing region" and the "self-complementary region") and they suggest that intramolecular base-pairing could occur between "the target hybridizing region and the self-complementary region and/or by base pairing between complementary sequences within the self-complementary region" (*See,* Agrawal et al. page 8, lines 34 to 35 and page 9, lines 1 to 3). Neither of these options describes a hairpin RNA within which all of the base-paired nucleotides are part of the target hybridizing region, such as that produced by constructs recited in the claims of the present invention.

Agrawal et al. state that the base-pairing within the self-complementary region renders the oligonucleotides resistant to nucleolytic degradation (*See,* Agrawal et al. page 8, lines 32 to 35). However, because the oligonucleotides of Agrawal et al. function via antisense mechanisms, the self-complementary oligonucleotides have to dissociate to expose the target hybridizing region to interact with the target sequence. Accordingly, the "self-complementary" region must dissociate in the presence of the target nucleic acid sequence. To do so, "the intermolecular base-paired structure formed by the hybrid between the target nucleic acid sequence and the target hybridizing region is more thermodynamically stable than the intramolecular base-paired structure formed by the self-complementary oligonucleotide." *See,* Agrawal et al. page 9, lines 12 to

17 of Agrawal et al. Although Agrawal et al. in one odd sentence mention that the intramolecular base-pairing could "involve every nucleotide of the oligonucleotide," a self-complementary oligonucleotide in which the entire target hybridizing region is involved with intramolecular base-pairing would dissociate less efficiently in the presence of the target sequence than a self-complementary oligonucleotide where only a portion of the target hybridizing region is involved in the intramolecular base-pairing. This would have been readily recognized by one of ordinary skill in the art at the time.

Consequently, one of skill in the art would not design a fully self-complementary oligonucleotide for the purpose of antisense-based inhibition of gene expression according to Agrawal et al. In fact, once the intramolecular base-pairing exceeds about 20 nucleotides in length, one of ordinary skill in the art would understand that the molecule would not disassociate at an acceptable rate under physiological conditions to effectively bind to the target. (*See*, Wallace R.B. et al., Nucleic Acids Res., 1979, **May 4, 2009 Exhibit 16**). Consistent with such understanding, Agrawal et al. disclose their "preferred embodiment" to have only "about 10 intramolecular base-pairs formed in the self-stabilized oligonucleotide" (*See* Agrawal et al. page 15, lines 23 to 26).

Of course, the pending claims herein require 20 to 30 nucleotides. Selecting a molecule where intramolecular base-pairing involves "every nucleotide of the" 20 to 30 nucleotides, would render the disclosure of Agrawal et al. unsatisfactory for its intended purpose and therefore

cannot be an obvious selection. *See, e.g., In re Gordon*, 733 F.2d 900; 221 U.S.P.Q. 1125 (Fed. Cir. 1984) (**May 4, 2009 Exhibit 17**).

The hairpin RNA produced by the claimed invention functions by a mechanism not disclosed by Agrawal et al. and is not subject to the same functional restrictions as the oligonucleotides of Agrawal et al. Importantly, this was not known prior to Applicants' invention, making the selection of a fully complementary hairpin RNA even less obvious.

The Examiner also referred to Gold et al. for a teaching of RNAs with stem loop structures. However, the RNAs taught by Gold et al. are used to determine nucleotide sequences that provide an optimal motif for targeted <u>protein</u> interaction. The RNAs are not used to specifically reduce gene expression. Furthermore, in the exemplified hairpins of Gold et al., the loop regions of the hairpins contain the "target regions" that are presumed to interact with the target proteins. This design differs from that of the Applicants' invention, where the "target region" is part of the stem of the hairpin. The sequences in the loop regions of the RNAs of Gold et al. are randomized in order to optimize the sequence for protein binding, while the sequences in the stem region are held static. This is in direct contrast to the Applicants' RNA hairpins, where the targeting regions, located in the stem region, may be varied per given target. Consequently, even if Fire et al. and Gold et al. could properly be combined, the combination of Fire et al. with Gold et al. would have led one of ordinary skill in the art to place the targeting region in

the "loop" of a hairpin, which would not result in a double-stranded RNA targeting region and would not lead to Applicants' invention. Additionally, the "loop" of Gold et al. most definitely does not read on the "stuffer" of Applicants' invention. The function of the "stuffer" is to allow the complementary regions of the RNA to efficiently base-pair with one another, while the "loop" of Gold et al. exists to present a single-stranded nucleotide binding site to a protein.

Thus, the combination of Fire et al., who do not explicitly claim double-stranded RNAs with a stuffer fragment, and Agrawal et al., who teach antisense oligonucleotides, and Gold et al., who teach nucleic acid ligands, would not lead one of ordinary skill in the art to produce a double-stranded DNA construct with a stuffer fragment that produces a double-stranded hairpin RNA for RNA interference. Neither Chatterjee et al., nor Kotin et al., teach the expression of double-stranded RNA.

c) *Length of the double-stranded RNA.* Fire et al. reported the successful use of double-stranded RNA that was 299 to 1033 nucleotides long. The minimum length of the double-stranded or hairpin RNA produced by the constructs of the claimed invention is 20 to 30 nucleotides long. Although one of ordinary skill in the art may have been educated from the teachings of references from the antisense art, such as those of Agrawal et al., where small, unduplexed oligonucleotides were used, there was no teaching prior to the filing of the subject application to support the notion that double-stranded RNA shorter than 299 nucleotides long could cause RNA interference. Thus,

the selection to reduce the size of the double-stranded RNA duplex by an order of magnitude was a great departure from what was reported by Fire et al. More importantly, the prior art provided no guidance to one of ordinary skill in the art prior to the filing of the subject application for predicting how such a selection would affect the ability of the DNA constructs to produce RNA to delay, repress or otherwise reduce the expression of a target gene in a mammalian cell.. *See,* Riggs Decl. ¶ 24.

d) *Change to mammalian cells.* Fire et al. described RNA interference in *C.elegans.* They were able to inhibit the gene expression of a specific target gene by exogenously delivering double-stranded RNA that was 299 to 1033 nucleotides long to *C. elegans* cells. Applicants' invention calls for reduction of gene expression in mammalian cells. At the time of the subject invention, it was known in the art that double-stranded RNA could cause a non-specific response in some mammalian cells that resulted in cytotoxicity. Based on this response in mammalian cells, it was thought that "[a] similar mode of action would not be suspected to occur in mammals" for RNA interference. (Wagner R.W. and Sun L., Nature, 1998, **May 4, 2009 Exhibit 18**). The prior art provided no guidance to one of ordinary skill in the art prior to the filing of the subject application for predicting the effects of how selecting mammalian cells would affect the ability of the DNA constructs to produce RNA to delay, repress or otherwise reduce the expression of a target gene in the mammalian cell. *See,* Riggs Decl. ¶ 25.

Uncertainties of the selections made by the present invention.

At the infancy of RNA interference, Applicants' invention was a significant deviation from what was known to have a RNA interference effect. At that time, the mechanism underlying the effects reported by Fire et al. was a mystery and one skilled in the art had no framework within which to even rationally consider, much less predict, what effect any given change would have on the observations reported by Fire et al. Substantial evidence from the relevant time indicates that it was impossible to predict the effects of any change to that system. Proceeding with the selections made by the inventors of the subject application was fraught with uncertainties; the selections introduced numerous variables that could have impacted the function of Applicants' invention. Yet, despite the uncertainties, Applicants proceeded contrary to the expectations of the time. *See,* Riggs Decl. ¶¶ 13 and 14.

i) The Selection of Endogenous Delivery Presented a Number of Unknowns.

- *It was unknown whether duplex RNA would get out of the nucleus*

Prior to Applicants' invention, the only knowledge in the art regarding the intracellular location where RNA interference was happening was taught by Fire et al., who disclosed that efficient gene silencing occurred when double-stranded RNA was injected into the body cavity or cytoplasm of *C. elegans*. Therefore, based on Fire et al., it would not be obvious that duplex RNA produced in the nucleus would efficiently translocate across the

nuclear membrane to elicit the same inhibition observed by Fire et al. It was unknown whether the canonical export machinery would recognize duplex RNA to promote its egress from the nucleus. It was also unknown whether any nuclear retention factors would bind the duplex RNA to prevent it from leaving the nucleus.

In the November 3, 2008 Office Action, the Examiner discusses that viral vectors could be used to introduce exogenous sequences into the genome of a cell. Kotin et al. generally discuss that AAV-based vectors could be used in human gene therapy. Chatterjee et al. use AAV vectors to express antisense RNA targeted to the 5' UTR of HIV. However, the production of antisense RNA in the nucleus is irrelevant because the claimed invention results in the production of duplex RNA in the nucleus. Chatterjee et al. did not demonstrate the production of duplex RNA in the nucleus. As already discussed, the characteristics of antisense RNA are not applicable to RNA interference. As Fire et al. admitted, "A simple antisense model" could not explain what they observed (Fire et al. Nature. 1998).

Nonetheless, antisense RNA may not even have to exit the nucleus to promote its inhibitory effects, which may include "transcriptional regulation, inhibition of splicing, inhibition of mRNA transport, and induction of mRNA instability" (Kumar M. and Carmichael G., Microbiol. Mol. Biol. Rev., 1998, **May 4, 2009 Exhibit 19**). In one example from the antisense RNA art, antisense RNA produced in the nucleus resulted in the nuclear retention of duplex RNA formed with the target sense strand (Kim S. and Wold B.J., Cell, 1985, **May 4, 2009 Exhibit 20**). This example is illustrative in two respects: (1) it demonstrates that antisense RNA does not have to leave the nucleus to function; (2)

it suggests that duplex RNA can get trapped in the nucleus. Consequently, the Examiner's argument that functional antisense RNA could be produced from an expression construct is irrelevant to the uncertainty of producing duplex RNA in the nucleus. Furthermore, one skilled in the art would have questioned whether duplex RNA could get out of the nucleus. *See,* Riggs Decl. ¶ 17.

- *It was unknown whether duplex RNA produced in the nucleus would be modified so as to make it ineffective for RNA interference*

The nucleus is a specialized compartment of the cell and contains factors that may only interact with macromolecules produced inside the nucleus. One example of such a factor is a nuclear double-stranded RNA dependent adenosine deaminase. In the nucleus, these enzymes target double-stranded RNA portions of duplexes and convert adenosine (A) to inosine (I), which makes the duplex unstable and may lead to unwinding and increased degradation (Kumar M. and Carmichael G., Microbiol. Mol. Biol. Rev., 1998, **May 4, 2009 Exhibit 19**). One of skill in the art would recognize that unwinding of the duplex would abrogate silencing function, based on the teaching of Fire et al. that the double-stranded character was important for function in RNA interference. *See, e.g.* Fire et al. Provisional, page 14, lines 22-25. Additionally, the incorporation of inosine in the RNA would decrease the stringency of the intramolecular base-pairing within the duplex, which could result in a heterogeneous collection of imperfect duplexes in the nucleus. Because one skilled in the art could not predict the effect that inosines would have on RNA interference, it would have been difficult to predict if RNA duplexes created in the nucleus could mediate gene silencing. *See,* Riggs Decl. ¶ 18.

- *The possibility of polyadenylation made it impossible to predict whether the claimed invention would successfully cause RNA interference*

Single-stranded messenger RNA precursors (pre-mRNA) that are produced in the nucleus are modified at their 3'terminus by the addition of a polyadenylation signal (poly-A tail) of ~200-250 adenine residues (Lodish et al., Molecular Cell Biology, c1999, **May 4, 2009 Exhibit 21**). At the time of the present invention, two proposed functions of the poly-A tail were: (1) to protect the transcript from degradation (Sachs A. and Wahle E. J. Biol. Chem. 1993, **May 4, 2009 Exhibit 22**); and (2) to stimulate transportation out of the nucleus (Huang Y. and Carmichael G., Mol. Cell. Biol., 1996, **May 4, 2009 Exhibit 23**). Therefore it was understood to be important for RNA produced in the nucleus to have a polyadenylation tail for protection and for transport out of the nucleus, and Applicants discuss the inclusion of a poly-A tail in the Provisional on page 22, lines 12 to 30. However, one skilled in the art would not have been able to predict the effect that a poly-A tail would have had on the ability of an RNA duplex to mediate RNA interference. If separate strands are transcribed, the poly-A tail would lead to a large, single-stranded overhang on both strands of the RNA duplex produced in the nucleus. Because the RNA duplexes of Fire et al. did not have a poly-A tail, it was not possible to predict how this structure would affect the RNA interference function of the RNA duplex resulting from the present invention. *See,* Riggs Decl. ¶ 20.

- *It was unknown whether binding of heterogeneous nuclear ribonucleoproteins (hnRNPs) would affect duplex formation, and consequently activity*

When RNA is produced in the nucleus, it is quickly bound by numerous heterogeneous nuclear ribonucleoproteins (hnRNPs). One function of these proteins is to promote the correct processing of endogenous pre-mRNA by preventing the formation of secondary structures, such as folding. (Lodish et al., Molecular Cell Biology, c1999, **May 4, 2009 Exhibit 21**). Thus, as RNA of the present invention is transcribed in the nucleus, hnRNPs could bind to the RNA and prevent it from folding to form a duplex. As stated above, Fire et al. considered the duplex structure of the injected RNA to be essential for RNA interference. Thus, the presence of hnRNPs in the nucleus could hinder the induction of RNA interference by inhibiting the formation of duplex RNA in the nucleus. *See,* Riggs Decl. ¶ 21.

Additionally, it was known that some hnRNPs from the nucleus remain associated with mRNA as it is translocated into the cytoplasm (Lodish et al., Molecular Cell Biology, c1999, **May 4, 2009 Exhibit 21**). Even if the RNA was able to assume a duplex structure, but the nuclear proteins remained bound to the RNA duplex when it encountered the silencing targets or unknown effectors in the cytoplasm, the bound duplex might not have been able to function properly to cause the interference. *See,* Riggs Decl. ¶ 22.

ii) Applicants' design calling for hairpin RNA presented additional unknowns

It was unknown whether the hairpin RNA would be susceptible to nucleus specific ribonucleases that would digest the hairpin RNA

Prior to Applicants' invention, a major concern with introducing RNA into cells was the degradation of that RNA. Assuming that the RNA of the present invention could form a hairpin in the nucleus, the hairpin would be expected to encounter nuclear double-stranded RNA ribonucleases (RNAses), such as RNAse III (Wu H. et al., J. Biol. Chem., 1998, **May 4, 2009 Exhibit 24**). These enzymes would be expected to specifically degrade the hairpin RNA in the nucleus, but would not be expected to degrade hairpin RNA directly introduced into the cytoplasm by Fire et al. If hairpin RNA structures were degraded in the nucleus, then RNA interference would not occur. *See,* Riggs Decl. ¶¶ 19 and 23.

iii) Applicants' design calling for shorter RNA duplexes presented yet more unknowns

It was unpredictable whether RNA duplexes an order of magnitude shorter than those exemplified in the prior art would be capable of repressing gene expression

The only report of RNA interference prior to Applicants' invention was by Fire et al. who showed that double-stranded RNA that was 299 nucleotides long was capable of efficiently causing RNA interference in *C. elegans.* Applicants' claims recite processes that require the use of DNA constructs designed to produce a dsRNA or a hairpin RNA which is only 20-30 nucleotides

long. Because the mechanism of RNA interference was unknown at the time, there was no indication prior to the filing of the subject application that the use of dsRNA or hairpin RNA an order of magnitude shorter than that shown to work by Fire et al. would cause the same result. Prior to Applicants' invention, one of ordinary skill in the art could not have predicted whether short hairpins would cause RNA interference. *See,* Riggs Decl. ¶ 24.

In fact, those of more than ordinary skill, e.g. the inventors listed on the Fire et al. Provisional, raised the issue in published statements. *See, e.g.,* Tabara H., Grishok A., and Mello C., Science 1998, **May 4, 2009 Exhibit 25.** ("In most genes, any RNA segment of about 200 to 1000 nucleotides or greater appears to be capable of inducing interference." "And controlled studies to determine the minimum length and the minimum sequence similarity to induce interference have yet to be reported and are likely to vary for different genes."). Subsequently, such beliefs from those of skill in the art prior to Applicants' invention have been shown to be incorrect and Applicants' inventive approach has been widely utilized.

Clearly, prior to the filing of the subject application, the consequence of decreasing the length of duplex RNA intended for gene silencing were unpredictable to those of ordinary as well as extraordinary skill in the nascent field. The nascent art in question was highly unpredictable.

iv) Expressing double-stranded RNA in mammalian cells presented even more unknowns

Prior to Applicants'invention, it was known in the art that double-stranded RNA could stimulate a non-specific mechanism in

some mammalian cells that led to the global inhibition of translation and transcript degradation. Double-stranded RNA can induce interferon production in mammalian cells, which causes the up-regulation of two double-stranded RNA response enzymes: RNA-regulated protein kinase (PKR) (also referred to as DAI) and 2′,5′ oligoadenylate synthetase (also called 2′,5′-oligo(A) polymerase). Direct activation of PKR by double-stranded RNA leads to the inhibition of translation (Clemens M., Int. J. Biochem. Cell. Biol., 1997, **May 4, 2009 Exhibit 26**). Double-stranded RNA also leads to the activation of 2′,5′ oligoadenylate synthetase, which catalyzes the production of 2′,5′ oligoadenylates. These molecules activate the ribonuclease RNAse L, which leads to the non-specific degradation of single-stranded RNA transcripts (Jacobs B.L. and Langland J.O., Virology, 1996, **May 4, 2009 Exhibit 27**). Studies described in the art at the time of the present invention maintained that double-stranded RNA had to be longer than 30 nucleotides to bind and activate either PKR or 2′,5′ oligoadenylate synthetase, and optimal binding occurred as the double-stranded RNA approached lengths of about 80 nucleotides. (Manche L. et al., Mol. Cell. Biol., 1992, **May 4, 2009 Exhibit 28**, and Minks M.A. et al., J. Biol. Chem, 1979, **May 4, 2009 Exhibit 29**). Thus, using the lengths of double-stranded RNA taught by Fire et al. to cause RNA interference in mammalian cells would have lead to a non-specific response resulting in cellular shutdown. However, it could not be predicted if reducing the length of the double-stranded RNA to below 30 nucleotides would result in RNA interference, as discussed above in Section (iii). *See,* Riggs Decl. ¶ 25.

Agrawal et al. and Chatterjee et al. is irrelevant prior art

The Examiner's proposal to combine teachings from antisense art with the Fire et al. Provisional is illogical in the face of the clear teaching in the Fire et al. Provisional that RNA interference operates by a distinct mechanism. *See, e.g.,* pages 2 to 5 of the Fire et al. Provisional. Consequently, the effects of such a combination cannot be predicted.

Nonetheless, the Examiner erroneously turned to the teachings of Agrawal et al. and Chatterjee et al. to provide evidence that knowledge of using antisense oligonucleotides to target viral RNA polymerase existed in the art at the time of the invention. However, the use of antisense oligonucleotides to perform this function has no predictive value on the ability of double-stranded RNA to inhibit viral RNA polymerase by the unknown mechanism of RNA interference. *See,* Riggs Decl. ¶¶ 8 and 26-27.

For instance, the Examiner references the teachings of Agrawal et al. to provide evidence that knowledge of hairpin RNAs with terminal loops existed in the art at the time of the invention. However, the function of the loops disclosed in that reference differs from that of the stuffer in the Applicants' invention. The loops of the antisense oligonucleotides in Agrawal et al. are present to protect the oligonucleotides from degradation in the cells. This differs from the purpose of the "stuffer fragment", which permits complementary base-pairing between the sense and the antisense strands of the hairpin, which are the regions that correspond to the target sequence.

Additionally, the Examiner cites a teaching from Chatterjee et

al. that antisense oligonucleotides targeting the "areas of critical viral RNA transcripts including the 5'-untranslated region, splice sites, and the polyadenylation signal have demonstrated significant antiviral activities." *See*, Chatterjee et al. column 3, lines 11 to 15. At the time, one proposed mechanism known in the art for antisense inhibition was a physical blockage of the translation machinery that resulted from the hybridization of the antisense oligonucleotide to the 5' untranslated region or the translation initiation codon. Because the mechanism of RNA interference was not known at the time, it was impossible to predict prior to Applicants' invention whether structural gene regions targeted to the 5' untranslated region would be effective.

### 3. The Obviousness Rejections Ignore the Perspective of One of Ordinary Skill in The Nascent Field of RNA Interference

Because RNA interference was an emerging art at the time Applicants' application was filed, it is imperative that the obviousness inquiry proceed based on what would have been obvious to a person of ordinary skill in the art at the time, not based on what is obvious to a judge, to an examiner, to a layman or to geniuses in the art then or now. The factors that are considered in determining the level of ordinary skill in the art include: 1) the education level of the inventor, 2) the types of problems encountered in the art, 3) the prior art solutions to those problems, 4) the rapidity with which innovations are made, 5) the sophistication of the technology, and 6) the educational level of the technical workers in the field. *Environmental Designs, Ltd. v. Union Oil Co.*, 713 F.2d 693, 697 (Fed. Cir. 1983), cert. denied, 464 U.S. 1043 (1984) (**May 4, 2009 Exhibit 30**). *See*, Riggs Decl. ¶ 4.

Applicants respectfully submit that as of March 20, 1998, a person of ordinary skill in the art related to the subject matter at issue herein would have been a person with a Ph.D. degree in microbial genetics, biochemistry, molecular biology or a related discipline with postdoctoral research experience in the field of recombinant DNA technology or a physician with equivalent educational and laboratory research experience in the same field. *See,* Riggs Decl. ¶ 4.

Individuals of ordinary skill at the time readily acknowledged that experiments designed to shed light on the possible mechanism of RNA interference "painted an even more mystifying picture" (Wagner R.W. and Sun L., Nature, 1998, **May 4, 2009 Exhibit 18**). Certainly those of ordinary skill in the art at the time would have recognized the multiple unknowns involved in trying to achieve RNA interference using endogenous delivery of hairpin RNA as discussed above. Because of this unpredictability in the nascent field at the time, one of ordinary skill could not have found the claimed invention obvious and the Examiner's assertions to the contrary are clearly erroneous.

**4. Substantial evidence of secondary considerations negates the alleged obviousness of the claimed invention**

i) The observation of unexpected results indicates that the invention could not have been obvious at the relevant time

The constructs recited by the pending claims herein have properties which could not have been predicted from the prior art. The attainment of unpredictable results is a clear

indication of non-obviousness. *See, e.g. KSR* at 1739-40. Despite the multitude of uncertainties associated with the claimed selection of elements, the selection disclosed in the subject application has been shown to be effective.

Applicants' claims are limited to a process requiring double-stranded DNA constructs comprising "a structural gene sequence comprising 20-30 consecutive nucleotides identical in sequence to a region of a target gene." The Examiner alleged that Applicants' claims are made obvious over Fire et al. because Fire et al. claim nucleotide sequences comprising at least 25 bases corresponding to a target gene. However, the range in Fire et al. is broad and encompasses "at least 25 bases" up to the length of the entire target gene. Nothing in Fire et al. teaches a preference for using a specific number of bases. Fire et al. exemplify using no less than 299 base pairs for RNA interference, thereby leading one of skill in the art to understand this to be the effective minimum amount of base pairs to cause RNA interference.

Applicants' claimed range only partially overlaps the extreme lower end of the Fire et al. range; a sub-range which was not tested by Fire et al. The situation is similar to that in *Atofina v. Great Lakes Chemical Corp.*, 441 F.3d 991, 999 (Fed. Cir. 2006) attached hereto as **Exhibit 5**, in which the Court held that a prior art reference that disclosed a range that only partially overlapped the claimed range did not invalidate the patent. There, the prior art disclosed a temperature of 100-500 °C, while the patent claimed the range 330-450 °C. The Court held that, "[g]iven the considerable difference between the claimed range and the range in the prior art, no reasonable fact finder could conclude that the prior art describes the claimed

range …." *See, Atofina*. As in *Atofina*, a person reading the prior art, which contains a disclosure of a broad partially overlapping range and exemplifies nothing below 299 base pairs, would not conclude that the art describes or enables the claimed range.

Additionally, Applicants' dependent claims 156-157 limit to 20 base pairs the length of consecutive nucleotides of a structural gene sequence that are identical in sequence to a region of a target gene. Such a feature of Applicants' invention is neither taught nor suggested by Fire et al. or any other prior art. Such a feature is also outside of the range which is purported to work by the Fire et al. Provisional.

Furthermore, the range of 20-30 bases is one of many elements selected by Applicants from the broad disclosure of the prior art. The combination of "20-30 bases" with "endogenous delivery" and "mammalian cells," amongst other elements, differentiates Applicants' claims from Fire et al., who generically described all conceivable numbers of bases, against all conceivable targets, delivered via all conceivable methods, to all conceivable cell types. From these broad ranges, Applicants proceeded to select specific elements, some of which were thought prior to Applicants' invention to be incompatible with RNA interference.

The efficacy of Applicants' approach came as a surprise to those skilled in the art, as illustrated by comments such as, "[m]ore surprising was the finding that DNA constructs encoding … blunt-ended duplexes with up to 29 base pairs were able to mediate RNA interference." Tuschl T., Nature Biotechnology, 2002, **May 4, 2009 Exhibit 32**. The constructs being referred to were RNA hairpins

that were produced from a double-stranded DNA construct with 27 or 29 base-pairs of "structural gene sequence" that was specific to a target gene. Paddison PJ et al., Genes & Development, 2002, **May 4, 2009 Exhibit 33**. Furthermore, in some cases, it was reported that endogenous delivery of hairpin RNA "dramatically reduced" expression of a target gene more efficiently than endogenous delivery of non-hairpin dsRNA. Yu J.Y. et al., Proc. Natl. Acad. Sci. USA 99, 2002, **May 4, 2009 Exhibit 34**.

It should be noted that Yu et al. used U6 promoters to produce hairpin RNA in the nucleus. It was well known in the art that the RNA produced in this manner was commonly retained in the nucleus. *See,* Noonberg SB et al., Nucleic Acids Research, 1994, **May 4, 2009 Exhibit 35**; and Good PD et al., Gene Therapy, 1997, **May 4, 2009 Exhibit 36**). This knowledge only accentuates the unpredictability of whether endogenous delivery of RNA would work, as discussed at length above. The understanding in the art at the relevant time was that the double-stranded RNA needed to be in the cytoplasm to cause RNA interference, not the nucleus. Thus, Noonberg et al. and Good et al. support Applicants' discussion that the results of the claimed selection could not have been predicted from teachings of the prior art.

In conclusion, prior to the filing of the subject application, the effectiveness of endogenously produced RNA was unexpected from the Fire et al. Provisional, alone or in combination with the secondary references from the prior art. The Examiner mistakenly ignored the objective criteria of nonobviousness.

ii) Evidence of objective criteria showing nonobviousness must also be considered when assessing patentability

Beyond the analysis of whether a proper *prima facie* case of obviousness is present, evidence of objective criteria showing nonobviousness must be considered. Specifically, skepticism of experts at the time is significant and respected objective evidence of nonobviousness. Such evidence is not cumulative in the obviousness analysis, but rather "constitutes independent evidence of nonobviousness." *Ortho-McNeil Pharmaceutical, Inc v. Mylan Laboratories, Inc.*, 520 F.3d 1358, 86 U.S.P.Q. 1196 (Fed Cir. 2008), citing *Catalina Lighting, Inc. v. Lamps Plus, Inc.*, 295 F.3d 1277, 1288 (Fed. Cir. 2002); *Pharmastem Therapeutics Inc. v. Viacell, Inc.*, 491 F.3d 1342; *Eli Lilly & Co. v. Zenith Goldline Pharms., Inc.*, 471 F.3d 1369 (**May 4, 2009 Exhibits 8 and 38 to 40**, respectively).

Thus, the skepticism of experts, including the skepticism of the inventors named on the Fire et al. Provisional, prior to Applicants' invention is highly probative and provides an "independent" basis for finding the pending claims patentable. The Examiner mistakenly ignored the objective criteria of nonobviousness.

a) *Skepticism in the art at the relevant time about the mechanism of RNA interference supports the patentability of the claimed invention*

As previously discussed, the mechanism of RNA interference was unknown at the time of the instant invention. Because the Fire et al. Letter to Nature, **May 4, 2009 Exhibit 15**, was the only report of RNA interference in the art at that time and the

results reported were so unexpected, there was at the time skepticism about the reported results themselves. *See,* Riggs Decl. ¶¶ 6-8. The following published statements reflect the impressions of those in the art regarding the difficulties and uncertainties associated with the initial RNA interference studies:

- "[T]he lack of a clear understanding of the critical requirements for interfering RNA led to a sporadic record of failure and partial success." Fire et al. Provisional, page 13, line 29 and page 14, line 1.

- Experiments designed to shed light on the possible mechanism of RNA "painted an even more mystifying picture." Wagner R.W. and Sun L., Nature, 1998, **May 4, 2009 Exhibit 18**.

It is apparent from these quotes that, at the relevant time, the RNA interference phenomenon was considered to be a mystery, characterized by numerous unknowns.

b) *Skepticism in the art at the relevant time about RNA interference in other eukaryotes supports the patentability of the claimed invention*

Because the Fire et al. Letter to Nature was the only report of RNA interference in the art at that time and the mechanism of RNA interference was unknown, there was also extreme skepticism concerning what sort of modifications the reported experiment would tolerate. *See,* Riggs Decl. ¶¶ 6-8 and 14. The following published statements reflect the impressions of those in the art

regarding the possibility of using RNA interference in systems other than *C. elegans*, such as other animals or mammals:

- "Whatever the mechanism might be, dsRNA-mediated inhibition of gene expression will provide a useful alternative for working out gene function in *C. elegans* and, maybe, in other animals and plants." (Emphasis added.) Wagner R.W. and Sun L., Nature, 1998, **May 4, 2009 Exhibit 18**.

- "A similar mode of action would not be suspected to occur in mammals." Wagner R.W. and Sun L., Nature, 1998, **May 4, 2009 Exhibit 18**.

- "Any gene-specific interference by dsRNA in PKR-proficient mammalian cells would be dependent on a transient lapse in the PKR response, or on a controlled level of dsRNA that was incapable of activating PKR." Montgomery M. and Fire A., Trends in Genetics, 1998, **May 4, 2009 Exhibit 41**.

These quotes represent, at the relevant time, the uncertainty of those skilled in the art as to whether the observed RNA interference would be applicable to other closely related organisms.

*c) Skepticism in the art at the relevant time about minimum length requirements of the targeting region of the double-stranded RNA further supports the patentability of the claimed invention*

The general skepticism of those skilled in the art was also relevant to other proposed modifications to the experimental system of Fire et al. The following published statements reflect the impressions of those in the art regarding the possibility of changing the length of the double-stranded RNA that caused RNA interference:

- "Controlled studies to determine the minimum length and the minimum sequence similarity to induce interference have yet to be reported and are likely to vary for different genes." Tabara H. et al., Science, 1998, **May 4, 2009 Exhibit 25.**

Taken alone or in concert, these quotes exemplify that there was significant skepticism at the relevant time about more minor modifications to the reported experimental system than the modifications resulting from the selection as claimed in the subject patent. Inventors of the claimed invention proceeded to face the multiple obstacles envisioned by many at the time and arrived at the claimed invention. Proceeding contrary to accepted wisdom at the time is, of course, inventive.

**III. Dependent Claims Recite Limitations That Are Nowhere Present In the Prior Art**

A number of Applicants' dependent claims recite limitations that are not found anywhere in the prior art. Dependent claims 147-149 limit the length of the stuffer fragment present in the double-stranded DNA constructs. Neither Fire et al., nor the other prior art references, mention stuffer fragments or limitations on stuffer fragment lengths. Additionally, dependent claims 156-157 limit to 20 base pairs the length of consecutive nucleotides of a structural gene sequence that are identical in sequence to a region of a target gene. Such a limitation is below the lowest limit of 25 base pairs mentioned by the Fire et al. Provisional and does not overlap with the disclosure of the Fire et al. Provisional. These claim limitations further differentiate Applicants' invention from the prior art and deserve proper consideration by the Examiner. In addition to the nonobviousness of Applicants' invention established in the arguments herein, these additional limitations further support the patentability of Applicants' invention.

The above discussion unambiguously shows that the pending claims are non-obvious in view of Fire et al., even when combined with Agrawal et al., Chatterjee et al., Gold et al. and Kotin et al., and should be allowed without further delay. Such action is respectfully requested. The obviousness rejections of the November 3, 2008 Office Action are inappropriate.

**Supplemental Information Disclosure Statement**

This Supplemental Information Disclosure Statement is being submitted as a supplement to the Supplemental Information Disclosure Statement Applicants submitted on May 4, 2009 in connection with the above-identified application. In accordance with the duty of disclosure under 37 C.F.R. § 1.56, Applicants direct the Examiner's attention to the following references which are listed on the Form PTO-1449 (Substitute) attached hereto as **Exhibit 6**.

According to 37 C.F.R. § 1.97(c), a Supplemental Information Disclosure Statement filed after the period specified in 37 C.F.R. § 1.97(b) shall be considered if accompanied by the fee set forth in 37 C.F.R. § 1.17(p) or a statement under 37 C.F.R. § 1.97(e). The required fee set forth in 37 C.F.R. § 1.17(p) is one hundred and eighty dollars ($180.00) and a check including this amount was enclosed with the May 4, 2009 submission.

Copies of the items listed below have been submitted to or provided by the United States Patent and Trademark Office in related patent applications. Applicants attach hereto as **Exhibit 7** a table listing locations where a copy of each listed reference may be found. Applicants respectfully direct the Examiner to the Image File Wrapper of the appropriate related application for a copy of the reference.

The Examiner is respectfully requested to make these references of record in the above-identified application by initialing and returning a copy of the enclosed Form PTO-1449 (Substitute).

1. Amendment submitted May 11, 2009 in connection with U.S. Serial No. 11/179,504, filed July 13, 2005;

2. Final Office Action issued August 13, 2009 in connection with U.S. Serial No. 11/179,504, filed July 13, 2005;

3. Final Office Action issued May 15, 2009 in connection with U.S. Serial No. 10/346,853, filed January 17, 2003;

4. Amendment submitted September 24, 2009 in connection with U.S. Serial No. 10/759,841, filed January 15, 2004;

5. Notice to the applicant regarding a non-compliant or non-responsive amendment issued September 4, 2009 in connection with U.S. Serial No. 10/821,726, filed April 8, 2004;

6. Communication issued May 21, 2009 in connection with U.S. Serial No. 11/218,999, filed September 2, 2005;

7. Response to Communication submitted June 22, 2009 in connection with U.S. Serial No. 11/218,999, filed September 2, 2005;

8. Supplemental Response To March 30, 2009 Amendment Filed In Response To September 30, 2008 Office Action filed August 4, 2009 in connection with U.S. Serial No. 11/218,999, filed September 2, 2005;

9. Amendment submitted July 15, 2009 in connection with U.S. Serial No. 10/646,070, filed July 13, 2005;

10. Amendment submitted May 11, 2009 in connection with

U.S. Serial No. 10/646,070, filed July 13, 2005;

11. Office Action, issued June 9, 2009 in connection with U.S. Serial No. 10/646,070, filed July 13, 2005;

12. Appeal Brief submitted July 27, 2009 in connection with Merged Reexamination Nos. 90/007,247 and 90/008,096, filed October 4, 2004 and May 18, 2006, respectively;

13. Amendment submitted July 15, 2009 in connection with U.S. Serial No. 10/571,384, filed June 1, 2006;

14. Office Action issued May 11, 2009 in connection with U.S. Serial No. 09/287,632, filed April 7, 1999;

15. Examiner Interview Summary Record (PTOL - 413) issued August 12, 2009 in connection with U.S. Serial No. 11/364,183, filed March 1, 2006.;

16. Notice to the applicant regarding a non-compliant or non-responsive amendment issued July 9, 2009 in connection with U.S. Serial No. 11/364,183, filed March 1, 2006.;

17. Supplemental Response or Supplemental Amendment submitted August 10, 2009 in connection with U.S. Serial No. 11/364,183, filed March 1, 2006.;

18. Office Action issued May 12, 2009 in connection with U.S. Serial No. 11/607,062, filed December 1, 2006;

19. Amendment submitted July 6, 2009 in connection with U.S. Serial No. 11/841,737, filed August 20, 2007;

20. Non-Final Rejection issued August 12, 2009 in connection with U.S. Serial No. 11/841,737, filed August 20, 2007;

21. Restriction Requirement issued May 4, 2009 in connection with U.S. Serial No. 11/841,737, filed August 20, 2007;

22. Giering J.C., et al. (2008) "Expression of shRNA from a tissue-specific pol II promoter is an effective and safe RNAi therapeutic," Mol Ther. 16(9):1630-6;

23. Ruiz F, Vayssié L, Klotz C, Sperling L, Madeddu L. (1998) "Homology-dependent gene silencing in Paramecium," Mol Biol Cell. 9(4):931-43.;

24. Sánchez Alvarado A, Newmark PA. (1999) "Double-stranded RNA specifically disrupts gene expression during planarian regeneration," Proc Natl Acad Sci U S A. 96(9):5049-54.; and

25. Song J., et al. (2004) "Poly(U) and polyadenylation termination signals are interchangeable for terminating the expression of shRNA from a pol II promoter," Biochem Biophys Res Commun. 323(2):573-8.

If a telephone interview would be of assistance in advancing prosecution of the subject application, Applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any other fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:

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10/7/09
Gary J. Gershik Date
Reg. No. 39,992

# EXHIBIT 1

**SUPPLEMENTAL AMENDMENT TO MAY 4, 2009 AMENDMENT**
**FILED IN RESPONSE TO NOVEMBER 3, 2008 OFFICE ACTION**

Submitted: October 7, 2009

**Serial No. 10/821,726**

Filed: April 8, 2004

Applicants: Michael Wayne Graham et al.